ENGINEERED INCOHERENT FEED FORWARD LOOP AND USES THEREOF

RELATED APPLICATION

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application No. 62/992,829 filed Mar. 20, 2020 which is incorporated by reference herein in its entirety.

FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with Government support under Grant No. MCB1840257 awarded by the National Science Foundation. The Government has certain rights in the invention.

BACKGROUND

[0003] In synthetic biology, methods for heterologous gene expression stabilization and disturbance rejection in mammalian cells remain limited. It is difficult to establish accurate, robust, and predictable control over heterologous gene expression using the known synthetic genetic circuits currently available.

SUMMARY

[0004] The present disclosure, at least in part, relates to an engineered incoherent feed forward loop (iFFL). An engineered incoherent feed forward loop, as used herein, refers to a class of network structure wherein an upstream node both activates and represses a downstream node through divergent branches (FIG. **1**H). In some embodiments, iFFLs are designed to provide robust perfect adaptation (RPA) of output expression to upstream disturbances, such as resource availability, off-target promoter interaction, and varying gene dosage (e.g., DNA copy number) (FIG. **1**G).

[0005] In some aspects, the iFFLs described herein comprise two transcription units: (i) a first transcription unit comprising a first promoter operably linked to a nucleic acid molecule encoding an endoribonuclease; and (ii) a second transcription unit comprising a second promoter operably linked to a nucleic acid molecule encoding an output molecule, and a endoribonuclease target site located within the 5' untranslated region (UTR) of the nucleic acid molecule encoding the output molecule, wherein the endoribonuclease is capable of cleaving the endoribonuclease target site on an RNA transcript expressed by the second transcription unit. In some embodiments, the first promoter and the second promoter are identical. In some embodiments, the first promoter and the second promoter share the same transcriptional resources.

[0006] In some embodiments, the first promoter and the second promoter are not identical. In some embodiments, the first promoter is at least 80% identical to the second promoter. An iFFL with non-identical promoters are useful, in some embodiments, to adapt the output molecule expression to the available copies of the first transcription unit and the second transcription unit, such as when there are different copy numbers of the first transcription unit and the second transcription unit.

[0007] In some embodiments, the endoribonuclease is a CRISPR-associated endoribonuclease. In some embodiments, the CRISPR-associated endoribonuclease is an endoribonuclease from the Cas6 family or the Cas13 family. In some embodiments, the CRISPR-associated endoribonuclease is CasE, Cas6, Csy4, Cse3, PspCas13b, RanCas13b, PguCas13b, or RfxCas13d.

[0008] In some embodiments, the first transcription unit further comprises at least one upstream open reading frame (uORF) located within the 5'UTR of the nucleotide sequence encoding the endoribonuclease. Such uORF are capable of regulating or fine tuning the expression of the endoribonucleases. Placement of different numbers of uORF within the 5'UTR of the nucleotide sequence encoding the endoribonuclease results in varying level of expression of the endoribonuclease. In some embodiments, the first transcription unit comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 uORFs. In some embodiments, the uORF comprises a nucleotide sequence of ACCATGGGTTGA (SEQ ID NO: 1).

[0009] In some embodiments, the first transcription unit and the second transcription unit are present on the same nucleic acid or on different nucleic acids. In some embodiments, the first transcription unit and the second transcription unit are present on the same vector or on different vectors.

[0010] In some embodiments, the first promoter and the second promoter are constitutive promoters, inducible promoters, or tissue specific promoters.

[0011] In some aspects, the present disclosure also provides a cell comprising the engineered incoherent feed forward loop described herein.

[0012] In some aspects, the present disclosure also provides a composition comprising the engineered incoherent feed forward loop or the cell described herein. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier.

[0013] In some aspects, the present disclosure also provides a method for delivering an output molecule to a subject in need thereof, the method comprising: delivering to the subject the engineered incoherent feed forward loop, the cell, or the composition described herein.

[0014] In some aspects, the present disclosure also provides a method for delivering an output molecule to a cell in need thereof, the method comprising: contacting the cell the engineered incoherent feed forward loop, the cell, or the composition described herein.

[0015] In some aspects, the present disclosure also provides a method for maintaining expression level of an output molecule to transcriptional disturbance in a subject in need thereof, the method comprising: delivering to the subject the engineered incoherent feed forward loop, the cell, or the composition. In some embodiments, the first transcription unit and the second transcription unit are delivered on the same nucleic acid. In some embodiments, the first transcription unit and the second transcription unit are delivered on the same vector. In some embodiments, the first transcription unit and the second transcription unit are delivered on different nucleic acids. In some embodiments, the first transcription unit and the second transcription unit are delivered on the different vectors. In some embodiments, the ratio between the first transcription unit and the second transcription unit is proportional.

BRIEF DESCRIPTION OF DRAWINGS

[0016] FIGS. **1**A-**1**H: iFFL-based approach for decoupling modules with shared limited resources. FIG. **1**A: A genetic module comprising a single constitutive transcription unit. Other competing modules place a load on the free